DNA QIAAMP EXTRACTION OF HAIR AND NAIL

A. SCOPE

This protocol employs the QIAamp DNA Mini Kit, Buffer X1, and DTT to extract DNA from hair and nail.

B. QUALITY CONTROL

B.1 Protective gloves, a mask, and a lab coat must be worn at all times when performing this procedure.

B.2 Each new QIAamp DNA Mini Kit lot must undergo quality control testing prior to extracting casework samples.

Biological material with known results along with a reagent control will be extracted using all the components of the kit undergoing quality control testing. The extracted material will be carried though the entire DNA analysis process. The results obtained from the known extracted sample must be as expected and good quality, as described in the GlobalFiler (DOC ID 12628) interpretation guidelines, for the kit to pass quality control testing. The quality control data will be placed into the critical reagent binder.

B.3 An analyst that dilutes the concentrated Buffers AW1 and AW2 prior to their initial use will be watched by a second individual from the Biology Unit to confirm correct preparation; this second individual can be an analyst, Investigative Assistant, etc. Both individuals will initial the bottle. In addition, the lot number and expiration date of the added ethanol will be recorded on the bottle.

B.4 See DOC ID 1835 to determine reagent expiration dates.

B.5 Buffer X1 and DTT will undergo quality control testing prior to being used in the extraction of casework samples.

Known hair or nail samples along with a reagent control will be extracted using the Buffer X1 undergoing quality control testing. The extracted samples will be carried through the entire DNA analysis process. The Buffer X1 will pass quality control testing when a good quality DNA profile, as described in the GlobalFiler (DOC ID 12628) interpretation guidelines, is obtained with the correct results. The quality control data will be placed into the critical reagent binder. DTT will be quality control

tested as described in DOC ID 1774. If the same lot number of powder DTT as previously quality control tested needs to be prepared, the analyst preparing the new batch of DTT will be watched by a second individual from the Biology Unit to confirm correct preparation. Both individuals will initial the box. Additional quality control testing is not required.

B.6 At least two reagent controls must be extracted along with a set of questioned samples.

C. SAFETY

C.1 Protective gloves, a lab coat, and a mask must be worn at all times when performing this procedure. Additionally, eye protection (e.g. safety glasses or a face shield) must be worn if this procedure is performed outside of a hood.

C.2 The sample preparation waste contains guanidine hydrochloride from Buffers AL and AW1, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilled, clean with water or ethanol.

C.3 All appropriate SDS sheets must be read prior to performing this procedure.

C.4 Treat all biological specimens as potentially infectious.

C.5 Distinguish all waste as general, biohazard, or sharps and discard appropriately.

D. REAGENTS, STANDARDS, AND CONTROLS

D.1 QIAamp DNA Mini Kit

D.1.1 Buffer AL
D.1.2 Proteinase K
D.1.3 Buffer AW1

Before using for the first time, add 125 mL ethanol (Absolute) to 95 mL AW1 concentrate

D.1.4 Buffer AW2

Before using for the first time, add 160 mL ethanol (Absolute) to 66 mL AW2 concentrate

D.1.5 Buffer AE

D.2 Absolute Ethanol (200 proof)

D.3 DTT, 1M Dithiothreitol, 5 mL

Dissolve 0.77 g dithiothreitol ($C_4H_{10}O_2S_2$, e.g. Sigma D-9779 molecular biology grade, 4°C.) in 5 mL sterile deionized water in a sterile, disposable plastic 15 mL tube or the original container, or add 6493 µL of deionized water to the bottle containing 1 g of the dithiothreitol. Store 100 µL aliquots in 0.5 mL microfuge tubes at approximately -20° C. Discard any unused portion of a thawed tube.

D.4 BUFFER X1

10mM Tris HCL, 10mM EDTA (RT), 100mM NaCl (RT), 2% SDS

Combine 1 mL Tris HCL 1M pH 8.0, 2 mL 0.5M EDTA pH 8.0, 20 mL NaCl 5M, and 20 mL SDS 10%, in 57 mL H_2O.

D.5 Bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner (Decontamination)

D.6 70% Reagent Alcohol (Decontamination)

D.7 Xylene

E. EQUIPMENT & SUPPLIES

E.1 Equipment

E.1.1 Scissors/Forceps
E.1.2 Microcentrifuge
E.1.3 Eppendorf ThermoMixer
E.1.4 Eppendorf Smartblock 1.5 mL
E.1.5 Eppendorf Smartblock 2.0 mL
E.1.6 Eppendorf ThermoTop
E.1.7 Pipettes
E.1.8 Vortexer
E.1.9 Hood (optional)
E.1.10 Diamond scribe
E.1.11 Scalpel

E.2 Supplies

E.2.1 Kimwipes
E.2.2 Microcentrifuge tubes
E.2.3 Spin baskets
E.2.4 Sterile aerosol resistant pipette tips
E.2.5 Microcentrifuge tube racks
E.2.6 Mask
E.2.7 Lab coat
E.2.8 Disposable gloves
E.2.9 Eye protection (e.g. safety glasses, face shield)
E.2.10 Post-it notes
E.2.11 Extraction sheet

F. PROCEDURE

F.1 If hair is not mounted proceed to step F.2. For mounted hairs, freeze the slide in an approximately -20°C freezer for 20 minutes. Remove the cover slip by prying it off using a scalpel. Alternatively, the cover slip may be removed by soaking the slide in xylene for several hours after cracking the cover slip. Using a pipette, wash away the mounting medium by squirting with xylene. Pick up the hair with sterile forceps and wash in a microcentrifuge tube containing ethanol (absolute); then wash in a microcentrifuge tube containing sterile, deionized water.

F.2 Remove the root end of the hair and place it in a labeled microcentrifuge tube or place the nail in a labeled microcentrifuge tube. Add 200 µL Buffer X1, 80 µL DTT and 2.5 µL Proteinase K.

F.3 Incubate at approximately 56°C with approximately 550 rpm mixing for at least 1 hour or until the sample is dissolved using a ThermoMixer. Invert the tube occasionally to disperse the sample (if necessary). Lysis time will vary depending on the size and density of the source material. This digest should go no longer than 24 hours. The incubation time must be recorded and can be documented as a start and end time or total incubation time.

F.4 Briefly centrifuge to remove drops from inside the lid. If a post-it note was utilized for hair root collection, use sterile forceps to remove the post-it note and place it in a spin basket. Place the basket back into the tube and centrifuge to collect the fluid remaining in the post-it note. Centrifuge the sample for 5 minutes at maximum speed. Add 200 µL AL Buffer and 200 µL ethanol (absolute). Vortex vigorously.

F.5 Briefly centrifuge to remove drops from inside the lid. Carefully remove liquid from the microcentrifuge tube and add to the labeled QIAamp spin column without wetting the rim. Close the cap and centrifuge at approximately **6,800 rcf (8,000 rpm)** for 1 minute.

F.6 Place the QIAamp spin column in a clean collection tube and discard the tube containing the filtrate. Carefully open the spin column and add 500 µL AW1 Buffer without wetting the rim. Close the cap and centrifuge at approximately **6,800 rcf (8,000 rpm)** for 1 minute.

F.7 Place the QIAamp spin column in a clean collection tube and discard the tube containing the filtrate. Carefully open the spin column and add 500 µL AW2 Buffer without wetting the rim. Close the cap and centrifuge at approximately **20,800 rcf (14,000 rpm)** for 3 minutes.

F.8 Continue with step F.10, or if there is AW2 Buffer carryover, perform step F.9.

F.9 (Optional): Place the QIAamp spin column in a new collection tube and discard the collection tube with the filtrate. Centrifuge at approximately **20,800 rcf (14,000 rpm)** for 1 minute.

F.10 Place the QIAamp spin column in a clean labeled microcentrifuge tube (this will be the final storage tube) and discard the tube containing the filtrate. Carefully open the spin column and add 50 to 100 µL of AE Buffer.

F.11 Incubate at room temperature for at least 1 minute. Centrifuge at approximately **6,800 rcf (8,000 rpm)** for 1 minute.

F.12 Quantitate (DOC ID's 1784 and 1785) the DNA and concentrate samples (DOC ID 1780) as necessary. Alternatively, samples may be concentrated prior to quantitation. Store sample extracts in the refrigerator when not in use. Sample extracts may be frozen for long-term storage.

G. INTERPRETATION GUIDELINES

Not applicable

H. REFERENCES

H.1 QIAamp DNA Mini and Blood Mini Handbook. Third Edition, 04/2010